Psychoneuroendocrinology 92 (2018) 57–65

Contents lists available at ScienceDirect

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen

# Chronic stress, hair cortisol and depression: A prospective and longitudinal study of medical internship

Stefanie E. Mayer[a,b,*], Nestor L. Lopez-Duran[a], Srijan Sen[b], James L. Abelson[b]

[a] Department of Psychology, University of Michigan, 530 Church Street, Arbor, MI, 48109, USA
[b] Department of Psychiatry, University of Michigan, 4250 Plymouth Rd, Ann Arbor, MI, 48109, USA

ARTICLE INFO

*Keywords:*
HPA
Hair cortisol
Chronic stress
Depression
Medical internship

ABSTRACT

*Background:* Stress plays a causal role in depression onset, perhaps via alteration of hypothalamic-pituitary-adrenal (HPA) axis functioning. HPA axis hyperactivity has been reported in depression, though inconsistently, and the nature of this relationship remains unclear, partly because cortisol measurement over time has been challenging. Development of hair cortisol assessment, a method that captures cortisol over prolonged periods of time, creates new possibilities. In this study, hair cortisol was incorporated into a prospective and longitudinal study of medical internship, stress and symptoms of depression. This provided a rare opportunity to 1) prospectively assess hair cortisol responses to stress, and 2) examine whether stress-induced changes in hair cortisol predict depressive symptom development.

*Methods:* Hair cortisol, depressive symptoms, and stress-relevant variables (work hours, sleep, perceived stress, mastery/control) were assessed in interns (n = 74; age 25–33) before and repeatedly throughout medical internship.

*Results:* Hair cortisol sharply increased with stressor onset, decreased as internship continued, and rose again at year's end. Depressive symptoms rose significantly during internship, but were not predicted by cortisol levels. Hair cortisol also did not correlate with increased stressor demands (work hours, sleep) or stress perceptions (perceived stress, mastery/control); but these variables did predict depressive symptoms.

*Discussion:* Hair cortisol and depressive responses increased with stress, but they were decoupled, following distinct trajectories that likely reflected different aspects of stress reactivity. While depressive symptoms correlated with stressor demands and stress perceptions, the longitudinal pattern of hair cortisol suggested that it responded to contextual features related to anticipation, novelty/familiarity, and social evaluative threat.

## 1. Introduction

Depression affects 16% of Americans at some point during their lives (Kessler et al., 2005) and represents the single largest contributor to global disability according to the World Health Organization (WHO, 2017). Life stress is the most common causal trigger of depression onset (Kendler et al., 1999). Understanding how life stress leads to depression can further our understanding of the disorder and inform prevention strategies. One potential pathway involves the hypothalamic-pituitary-adrenal (HPA) axis and its end product cortisol (Taylor et al., 1997). HPA axis hyperactivity has been linked with depression (reviewed in Nemeroff and Vale, 2005), but variations exist (e.g., depending on patient and clinical factors; Lamers et al., 2013; Stetler and Miller, 2011) and the temporal nature of this relationship is still unclear. There is some evidence that HPA axis dysregulation may precede depression (Adam et al., 2010; Harris et al., 2000), but interactions with chronic stress exposure are rarely examined.

The HPA axis is a complex system that is shaped by and interacts with psychosocial (Levine, 2000), contextual (Gunnar et al., 2009), genetic (Gotlib et al., 2008), and developmental factors (Tyrka et al., 2008). This system helps us respond to acute stress, but it also undergoes long-term changes in response to repeated stress experiences. These longer-term alterations may be particularly relevant in the etiology of depression (Ehlert et al., 2001). Tracking HPA activity over time holds the potential to illuminate its role in depression. However, quantification of longer-term HPA axis activity has been notoriously difficult as traditional cortisol measures in blood, saliva, or urine only capture activity over a period of minutes to hours and are sensitive to numerous confounding variables (reviewed in Russell et al., 2012). These methodological challenges have hampered efforts to understand the links between stress exposure, longer-term HPA activity, and depression onset.

* Corresponding author: Department of Psychiatry, University of California San Francisco, 3333 California St, Suite 465, San Francisco, CA, 94118, USA.
*E-mail addresses:* Stefanie.Mayer@ucsf.edu (S.E. Mayer), nestorl@umich.edu (N.L. Lopez-Duran), srijan@med.umich.edu (S. Sen), jabelson@med.umich.edu (J.L. Abelson).

https://doi.org/10.1016/j.psyneuen.2018.03.020
Received 11 October 2017; Received in revised form 21 March 2018; Accepted 26 March 2018

Development of hair cortisol assessment, a method that quantifies cumulative cortisol production over prolonged periods of time, creates new research possibilities. It has been validated in clinical and non-clinical contexts to reflect systemic long-term cortisol exposure (retrospectively, up to 6 months; Gow et al., 2010). It also may allow us to assess stress-induced changes in cortisol exposure longitudinally, over months to years, which could provide insights into the role of HPA axis functioning in depression. Cross-sectional studies have shown elevated hair cortisol in stressed populations (Stalder et al., 2017; Staufenbiel et al., 2013), but prospective studies that assess within-person *changes* in hair cortisol accumulation in the context of long-term stress exposure are lacking. Some cross-sectional studies have also shown positive associations between hair cortisol and clinically diagnosed depressed patients (mostly inpatients; Dettenborn et al., 2012) as well as between hair cortisol and self-reported depressive symptoms in non-clinical community samples (Abell et al., 2016; Faresjo et al., 2013; Stalder et al., 2014; Wikenius et al., 2016). However, the first meta-analytic review, which included primarily cross-sectional studies, did not show consistent links with self-reported depressiveness (Stalder et al., 2017). Prospective stress designs are needed to determine the temporal relationships between chronic stress, long-term HPA axis activity, and depressive symptoms. Because stress, by its nature, is unpredictable and heterogeneous, such studies have been difficult.

Medical internship—the first year of professional clinical training for physicians following medical school graduation—provides a naturalistic chronic stress paradigm. It is a time of high stress (Butterfield, 1988) and meta-analytic evidence (54 studies; n = 17 560 resident physicians) estimates an overall prevalence of depression during residency of 28.8%, ranging from 20.9% to 43.2% (Mata et al., 2015). Medical internship allows for prospective tracking of perceived and experienced stress and the development of depression, starting before stressor onset, and with longitudinal follow-up throughout the 12 months of internship exposure. The somewhat homogenous sample facing a relatively "standardized" stressor may provide a paradigm with reduced "noise" that might otherwise obscure linkages.

The combination of hair cortisol technology and the internship model allows us, for the first time, to prospectively assess how chronic stress exposure affects HPA axis functioning and determine whether stress-induced changes in HPA axis functioning are linked to depressive symptom development. Based on previous cross-sectional studies, we hypothesized that 1) hair cortisol levels will significantly increase with internship stress and 2) the increase in cortisol levels will associate with an increase in depressive symptoms.

## 2. Material and methods

### 2.1. Participants

The study was part of an ongoing longitudinal study of depression during medical internship (Sen et al., 2010). Participants were recruited from graduating University of Michigan Medical School students who matched to attend internship within 50 miles of Ann Arbor to allow in-person collection of hair samples and to control for other pre-internship stressors (e.g., moving). Participants were required to have a minimum hair length of 1 cm. They signed written consent and were paid $350 for study participation. We collected data from residency cohorts over a 4-year period from 2012 to 2015 (2012: n = 18, 2013: n = 23, 2014: n = 14, 2015: n = 19), yielding a final sample of 74 participants. The study was approved by our local Institutional Review Board (IRB).

### 2.2. Procedures and measures

#### 2.2.1. Hair assessment

Hair samples were collected 1–2 months prior to internship start (pre-internship) and at the four-, eight- and twelve-month time points during internship year, following guidelines from the Society of Hair Testing (Cooper et al., 2012). At each hair collection time point, 2–3 hair samples were cut with scissors from the posterior vertex region of the head (cut close to the scalp without pulling hair; all collected hair samples were analyzed). After hair collection, samples were wrapped in aluminum foil and stored at room temperature (Gow et al., 2010). Annual collections were analyzed in batches, such that when the last hair sample was obtained at the end of a given internship year (cohort), all samples of that year were sent to Dr. Kirschbaum's laboratory at the Dresden University.

Starting at the scalp-near end, samples were cut into two 2–cm segments for analysis (where length permitted; mean segment weight was 5.5 mg ± 0.5 mg). Hair growth rates vary between individuals (Schütz et al., 1993), but a rate of 1 cm/month has been generally accepted in the literature for a 1–cm hair segment (Schütz et al., 1993; Wennig, 2000). The first 2–cm segment (Segment 1) thus reflects total cortisol production over the 2 months prior to the collection time point; the next 2–cm segment (Segment 2) reflects secretion during months 2–4 before the collection time point. When we subsequently refer to hair cortisol levels at a specific time point, these actually reflect cortisol secretion over the prior months as described above. Hair samples were assayed for cortisol using a validated, commercially available immunoassay with chemiluminescent detection (procedures are described in more detail in Stalder et al., 2012).

#### 2.2.2. Self-report measures

Participants provided *socio-demographic* (sex, age, ethnicity, marital status, having a child, medical specialty), *health-related* (Body Mass Index—BMI, smoking, antidepressant use, oral contraceptive use, personal history of depression, family history of depression, stressful life events in the past 3 months), and *hair-related information* (hair color, use of hair products, hair coloring/dying/bleaching/perm, weekly hair washing frequency).

Self-reported *depressive symptoms* in the past 2 weeks were assessed prior to internship start and at three-month intervals during internship using the 9-item Patient Health Questionnaire (PHQ-9; Kroenke et al., 2001). *Perceived stress* and *sense of mastery/control* were measured prior to internship start and at four-month intervals during internship using the 10-item Perceived Stress Scale (PSS; Roberti et al., 2006) and Pearlin's 7-item Mastery Scale (Pearlin and Schooler, 1978), respectively (only available for cohorts 2013–2015). Other *internship information* (e.g., sleep hours/night in the past week, weekly work hours, days off in the past month) was also collected at pre-internship and during quarterly assessments.

### 2.3. Statistical analyses

#### 2.3.1. Data preparations

No outliers were excluded from analyses to avoid data loss, but extreme hair cortisol values at the upper 5% of the distribution were winsorized (set at the 95 percentile value) to reduce their impact on data analyses (Adam and Kumari, 2009; Wilcox, 1998). The winsorized hair cortisol raw data is presented in Fig. 1. Hair cortisol values and depressive symptoms (PHQ-9 scores) were log transformed to improve skewness and kurtosis. Hair cortisol concentrations between the first and the second 2-cm segments were highly correlated, but not identical (BL: $r = 0.875$, 4-months: $r = 0.912$; 8-months: $r = 0.819$; 12-months: $r = 0.715$). We analyzed both segments to examine hair cortisol concentrations throughout the entire internship year, testing links with stress exposure, psychosocial stress, and depressive symptoms. Generally, correlations of hair cortisol values between time points ranged from $r = 0.4$ to $r = 0.9$, suggesting both intra-individual stability, but also individual variability over time.

There is some loss of cortisol signal over time (reflected in declining concentrations farther from the scalp), possibly due to wash-out effects (Kirschbaum et al., 2009). Three independent, published samples report cortisol decline rates per 1-cm hair segment of 2.5 pg/mg (Kirschbaum

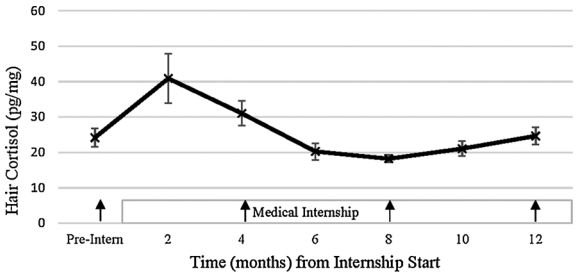


**Fig. 1.** Mean ( ± SE) of hair cortisol levels (winsorized raw data) as a function of time (months) from medical internship start. Arrows indicate collection of a 4-cm hair segment, which was then cut into two 2-cm hair segments. Hair cortisol levels at pre-internship, 2, 4, 6, 8, 10, and 12 months thus reflect total concentrations over the previous approximately 2-months' time interval.

et al., 2009), 2.7 ± 0.3 pg/mg (Gao et al., 2010), and 2.9 ± 0.6 pg/mg (Xie et al., 2011). These rates are remarkably consistent, suggesting that hair cortisol decline can be accounted for; adjusting for this decline is important for comparisons across segments. Based on Gao (2010), we estimated that hair cortisol values for the second 2–cm segment would be about 16% lower than those in the first 2-cm segment due to these "wash-out" effects. To more accurately compare across segments, we adjusted for this signal loss along the hair shaft and conservatively increased Segment 2 values by 10%.

Missing hair cortisol data at each assessment time point were low (0% at pre-internship; 8% at 4 months; 4% at 8 months; 11% at 12 months). However, at a given assessment time point, we could not obtain a full 4–cm hair sample from every participant. Male participants often had insufficient hair length to obtain a second 2–cm segment, which resulted in significant missing Segment 2 data (31%), yielding overall missing data of 18% (50% of participants had complete hair cortisol data for every time point and both hair segments). We imputed missing hair cortisol data using a Fully Conditional Specification Method Iterations—an iterative Markov Chain Monte Carlo (MCMC) method. Imputed hair cortisol values did not differ from non-imputed data (all $p$ values $> 0.80$, except hair cortisol at 10 months, $p = 0.16$). Imputing the missing data allowed us to obtain less biased estimates of population parameters than modeling the incomplete dataset (Lee and Carlin, 2010). However, analyses without the additional manipulations (imputing missing data and adjusting Segment 2 values) yielded identical results.

### 2.3.2. Statistical analysis

As a first step, we assessed hair cortisol responses to internship stress by using repeated measures analysis of variance (RM-ANOVA) within a mixed model framework. The time variable was coded as months from internship start (starting at 0 months, which was the reference category). Hair cortisol levels were also compared between time points using Bonferroni correction for multiple comparisons. Since we were not necessarily interested in hair cortisol levels at specific time points, but rather in overall hair cortisol trajectories throughout internship, we conducted the main analyses using growth curve modeling.

We examined hair cortisol and depressive responses to internship stress using multilevel growth curve modeling (GCM). The time

**Table 1**
Self-Reported Socio-Demographic, Health-, and Hair-Related Information Prior to Internship Start (Mean ± SD or Valid Percentage).

| Pre-Internship | Variable | Variable – Subcategory | Statistics |
|---|---|---|---|
| Socio-Demographic | Cohort | 2012 | 24% |
| | | 2013 | 31% |
| | | 2014 | 19% |
| | | 2015 | 26% |
| | Sex (percent female) | | 56% |
| | Age (years) | | 27.41 ± 2.36 |
| | Ethnicity | Caucasian | 80% |
| | | African American | 1% |
| | | Asian | 16% |
| | | Other | 3% |
| | Marital Status | Single | 61% |
| | | Engaged | 10% |
| | | Married | 29% |
| | Having Children (percent yes) | Yes | 11% |
| | Medical Specialty | Internal Medicine | 13% |
| | | Surgery | 6% |
| | | Obstetrics/Gynecology | 9% |
| | | Pediatrics | 10% |
| | | Psychiatry | 3% |
| | | Emergency Medicine | 9% |
| | | Med/Peds | 3% |
| | | Family Practice | 6% |
| | | Other | 37% |
| | | Transitional | 6% |
| Health-Related | Smoking | | 0% |
| | Body-Mass-Index (BMI) | | 23.02 ± 3.51 |
| | Oral Contraceptive Use (percent yes) | | 25% |
| | Antidepressant Medication Use (percent yes) | | 10% |
| | Personal History of Depression (percent yes) | | 53% |
| | Family History of Depression (percent yes) | | 60% |
| | Stressful Life Event (> = 1 event in past 3 months) | | 26% |
| Hair-Related | Natural Hair Color | Brown | 62% |
| | | Black | 15% |
| | | Blonde/Red | 23% |
| | Hair Treatment | Use of Hair Products (Gel, Spray, Wax) | 15% |
| | | Hair Coloring/Dying/Bleaching/Perm | 8% |
| | | No Hair Treatment | 80% |
| | Hair Washing Frequency (per week) | | 5.92 ± 1.89 |

**Table 2**
Unconditional and Covariate-Adjusted Models Predicting Hair Cortisol Trajectory.

| Model | Parameter | Estimate | Std. Error | df | t | Sig. |
|---|---|---|---|---|---|---|
| Unconditional Model | Intercept | 1.2645 | 0.0393 | 165 | 32.14 | < 0.001 |
| | Time | 0.1171 | 0.0188 | 440 | 6.24 | < 0.001 |
| | Time * Time | −0.0288 | 0.0038 | 441 | −7.49 | < 0.001 |
| | Time * Time * Time | 0.0016 | 0.0002 | 441 | 7.64 | < 0.001 |
| Model Adjusted for Covariates | Intercept | 1.3356 | 0.0847 | 116 | 15.78 | < 0.001 |
| | Time | 0.0763 | 0.0376 | 368 | 2.03 | 0.043 |
| | Time * Time | −0.0215 | 0.0077 | 369 | −2.79 | 0.005 |
| | Time * Time * Time | 0.0013 | 0.0004 | 369 | 3.04 | 0.003 |
| | Age | 0.0401 | 0.0169 | 163 | 2.38 | 0.019 |
| | Age * Time | 0.0111 | 0.0089 | 368 | 1.25 | 0.213 |
| | Age * Time * Time | −0.0036 | 0.0018 | 369 | −1.97 | 0.050 |
| | Age * Time * Time * Time | 0.0002 | 0.0001 | 369 | 2.22 | 0.027 |
| | Single | 0.1603 | 0.0650 | 57 | 2.47 | 0.017 |
| | Engaged | 0.1747 | 0.1123 | 57 | 1.55 | 0.126 |
| | Married | 0 | 0 | | | |
| | Cohort 2012 | −0.3998 | 0.1023 | 174 | −3.91 | < 0.001 |
| | Cohort 2013 | −0.2645 | 0.0986 | 175 | −2.68 | 0.008 |
| | Cohort 2014 | 0.0006 | 0.1170 | 177 | 0.01 | 0.996 |
| | Cohort 2015 | 0 | 0 | | | |
| | Cohort 2012 * Time | −0.0229 | 0.0556 | 368 | −0.41 | 0.681 |
| | Cohort 2013 * Time | 0.0917 | 0.0537 | 368 | 1.71 | 0.089 |
| | Cohort 2014 * Time | 0.0979 | 0.0640 | 368 | 1.53 | 0.127 |
| | Cohort 2015 * Time | 0 | 0 | | | |
| | Cohort 2012 * Time * Time | 0.0047 | 0.0114 | 369 | 0.41 | 0.679 |
| | Cohort 2013 * Time * Time | −0.0147 | 0.0110 | 369 | −1.34 | 0.182 |
| | Cohort 2014 * Time * Time | −0.0243 | 0.0131 | 369 | −1.85 | 0.064 |
| | Cohort 2015 * Time * Time | 0 | 0 | | | |
| | Cohort 2012 * Time * Time * Time | −0.0003 | 0.0006 | 369 | −0.43 | 0.665 |
| | Cohort 2013 * Time * Time * Time | 0.0006 | 0.0006 | 369 | 1.03 | 0.304 |
| | Cohort 2014 * Time * Time * Time | 0.0014 | 0.0007 | 369 | 1.92 | 0.056 |
| | Cohort 2015 * Time * Time * Time | 0 | 0 | | | |

Note: Dependent Variable: Hair cortisol (log transformed).

variable was coded as months from internship start. The unconditional model included an intercept (segment 1 pre-internship hair sample/baseline depressive symptom score), and fixed effects of time that modeled reactivity. Random intercepts were included in the model, allowing different participants to have different levels prior to internship. Random coefficients for time effects were also considered if appropriate. Restricted maximum likelihood estimates (REML) of parameters (SPSS MIXED command) were computed and an unstructured covariance structure was modeled for the random effect(s). Repeated errors associated with the same individuals were allowed to have an autoregressive covariance structure. Analyses also controlled for fixed effects of potential confounding variables. Continuous predictors were mean centered. We also examined relationships using Pearson Product Moment Correlations.

## 3. Results

### 3.1. Descriptive statistics

Descriptive statistics of sociodemographic, health- and hair-related information are displayed in Table 1. Participants (56% female) were between age 25 and 33, and majority Caucasian (80%), single (61%), and without children (89%). They had diverse medical specialties (e.g., 13% internal medicine, 6% surgery, 9% obstetrics/gynecology, 10% pediatrics), were non-smokers (before and throughout internship), and had healthy BMI scores (M ± SD = 23 ± 3.5 $kg/m^2$) as reflected in Center for Disease Control and Prevention standards. About 25% of the sample used oral contraceptives before internship (35% during internship). About 10% indicated using antidepressant medication before internship (15% during internship). More than half reported a personal (53%) and/or family (first degree relative) history of depression (60%). About one fourth of participants indicated having at least one major life event in the past three months before internship start (e.g., getting married, having a child, death of a family member, financial loss, physical assault). The majority of participants had natural brown hair (62%) and did not use any hair treatment (80%; no use of hair products or coloring/dying/bleaching/perm). Participants washed their hair on average 6 times a week.

During their internship year, participants worked an average of 62 h per week (SD = 12.47) and had on average 6 days off in the past month as indicated in quarterly surveys. They slept on average one hour/night less during internship (M ± SD = 6.62 ± 0.86), relative to pre-internship (M ± SD = 7.49 ± 1.08, $t(52) = 5.58$, $p < 0.001$). The experience was subjectively stressful, with an increase from pre-internship to 4 months in perceived stress (M ± SD = 10.96 ± 5.36, increasing to 13.83 ± 5.21, $t(50) = -4.12$, $p < 0.001$) and a sustained elevation over baseline throughout internship (M ± SD = 12.63 ± 4.89, $t(54) = -2.47$, $p = 0.017$). Only 3.6% of interns endorsed being highly stressed prior to internship, but 26% did so (PSS ≥ 20) at least once during internship.

### 3.2. Hair cortisol responses to internship stress

Repeated measures within a mixed model framework showed that hair cortisol levels significantly changed over time, $F(6, 253) = 10.47$, $p < 0.001$. Estimates of fixed effects showed that hair cortisol concentrations at 2 months (reflecting cumulative concentrations during the initial 2 months of internship), $b = 0.1799$, $p < 0.001$, and 4 months, $b = 0.0938$, $p = 0.016$, were elevated relative to pre-internship levels (0 months). Hair cortisol concentrations at 6 and 8 months did not differ from pre-internship levels ($p = 0.455$, $p = 0.109$, respectively). Notably, pre-internship hair cortisol levels (0 months) were elevated compared to internship levels at 10 months, $b = -0.0911$, $p = 0.011$, yet comparable to hair cortisol levels at 12 months, $b = 0.0192$, $p = 0.524$. Bonferroni corrected multiple comparisons between time points showed that following an initial hair cortisol increase

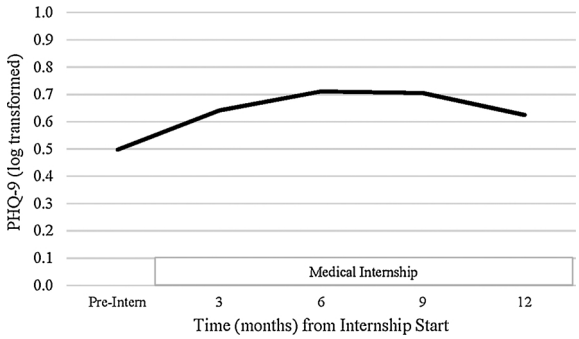


**Fig. 2.** Estimated depressive symptom trajectory (log transformed) in response to medical internship as a function of time (months) from internship start. PHQ-9 = 9-Item Patient Health Questionnaire. The graph shows that depressive symptoms increased with the onset of internship stress. Depressive symptoms remained elevated throughout internship.

in response to internship stress (0–2 months: $p < 0.001$), hair cortisol levels remained elevated at 4 months (2 vs. 4 months: $p = 0.094$), but then decreased from 4 to 6 months ($p = 0.001$), with no further significant changes (6 vs. 8 months: $p = 1.00$, 8 vs. 10 months: $p = 1.00$) until hair cortisol levels rose again from 10 months until 12 months at the end of internship, $p = 0.006$.

We also examined hair cortisol responses to internship stress using Growth Curve Modeling (GCM). A cubic trajectory represented the best fit for the data (lowest AIC; linear model AIC = 89.13; quadratic model AIC = 101.52; cubic model AIC = 61.79). Specifically, hair cortisol initially increased in response to internship stress (time $b = 0.1171$, $p < 0.001$), followed by a decline in hair cortisol as internship continued (time$^2$ $b = -0.0288$, $p < 0.001$), and a deceleration of this decline towards the end of internship (time$^3$ $b = 0.0016$, $p < 0.001$; see Table 2 Unconditional Model).

We also examined the impact of sociodemographic, health-, and hair-related covariates on hair cortisol trajectory. The year of medical internship (cohort) impacted hair cortisol levels, such that the earlier cohorts 2012 and 2013 had lower pre-internship hair cortisol levels compared to the last cohort 2015, intercept, $b = -0.4137$, $p < 0.001$, intercept, $b = -0.2498$, $p = 0.007$, respectively. The 2013 cohort also had a steeper linear increase (and marginally greater decrease) compared to cohort 2015, time, $b = 0.1040$, $p = 0.038$, time$^2$ $b = -0.0173$, $p = 0.093$, time$^3$ $b = 0.0007$, $p = 0.181$. Older age yielded more pronounced (reactive) quadratic and cubic trajectories, intercept, $b = 0.0202$, $p = 0.284$, time, $b = 0.0123$, $p = 0.161$, time$^2$ $b = -0.0036$, $p = 0.044$, time$^3$ $b = 0.0002$, $p = 0.027$. Being single, compared to being married, was associated with elevated pre-internship hair cortisol, intercept, $b = 0.2043$, $p = 0.028$, but had no effect on hair cortisol trajectory from pre-internship (all $ps > 0.20$). Sex, ethnicity (Caucasian vs. non-Caucasian), and having a child did not significantly impact hair cortisol responses (all $ps > 0.10$). Physical and mental health baseline variables (BMI, personal history of depression, family history of depression, stressful life events) also did not impact hair cortisol (all $ps > 0.05$). Medication intake throughout the study period did not impact cortisol trajectory (intercept $b = -0.0904$, $p = 0.255$, time, $b = -0.0487$, $p = 0.199$, time$^2$ $b = 0.0113$, $p = 0.146$, time$^3$ $b = -0.0006$, $p = 0.172$), nor did antidepressant use when examined separately (intercept $b = -0.0959$, $p = 0.388$, time, $b = -0.0189$, $p = 0.720$, time$^2$ $b = 0.0119$, $p = 0.272$, time$^3$ $b = -0.0008$, $p = 0.170$), or hormonal contraceptive use (intercept $b = -0.0170$, $p = 0.835$, time, $b = 0.0450$, $p = 0.245$, time$^2$ $b = -0.0095$, $p = 0.229$, time$^3$ $b = 0.0004$, $p = 0.312$). Mean days off in the past month (as indicated in quarterly surveys) also did not predict hair cortisol trajectory during internship, time, $b = 0.0086$, $p = 0.433$, time$^2$ $b = -0.0016$, $p = 0.484$, time$^3$ $b = 0.00007$, $p = 0.561$. We also examined the impact of hair-related variables on hair cortisol levels. Natural hair color or any hair treatment during the study period, including use of hair products or coloring/dying/bleaching/perm did not impact hair cortisol (all $ps > 0.20$). Greater weekly hair washing frequency throughout the study period was significantly related to lower pre-internship hair cortisol levels (intercept $b = -0.0522$, $p = 0.022$) without effects on hair cortisol trajectory during internship (all $ps > 0.20$). When entering all significant covariate effects into a single adjusted model, hair washing frequency no longer had a significant impact on pre-internship hair cortisol levels (intercept, $b = -0.0106$, $p = 0.536$). We subsequently only controlled for age, marital status, and cohort effects. The final covariate-adjusted model (see Table 2) showed that the cubic hair cortisol trajectory remained, showing an initial rise in cortisol with internship onset, a decrease as internship continued, and a late rise towards the end, prior to the start of the second residency year.

### 3.3. Depressive symptom responses to internship stress

Depressive symptoms (assessed by PHQ-9) and perceived stress ratings were highly correlated during internship ($r(51) = 0.677$, $p < 0.001$). Consistent with previous reports, depressive symptoms increased significantly from before internship (M ± SD = 2.78 ± 2.94) to 3 months (M ± SD = 5.03 ± 4.14, $p < 0.001$), and remained elevated over baseline throughout internship (6 months: M ± SD = 4.77 ± 3.42, $p < 0.001$; 9 months: 5.22 ± 4.36, $p < 0.001$; 12 months: 4.67 ± 4.51, $p = 0.001$). One third (33.3%) of interns reported at least moderate depressive symptoms (PHQ ≥ 10) at least once during internship.

This pattern was confirmed using Growth Curve Modeling, where a quadratic depressive symptom trajectory was the best fit for the data (lowest AIC; linear model AIC = 135.14; quadratic model AIC = 119.95; cubic model AIC = 132.20; time $b = 0.0605$, $p < 0.001$; time$^2$ $b = -0.0042$, $p < 0.001$; see Fig. 2).

We examined the impact of socio-demographic (cohort, sex, age, ethnicity, marital status, having a child) and health-related variables (BMI, medication intake throughout the study period, antidepressant use, oral contraceptive use, personal history of depression, family history of depression, stressful life events, and mean days off in the past month) on depressive symptom trajectory. Only the following had statistically significant effects on depressive symptom trajectory: There were cohort effects in depressive symptoms, such that the 2012 cohort had lower depressive symptoms at pre-internship, intercept $b = -0.2701$, $p = 0.011$, which yielded a more reactive depressive symptoms trajectory (steeper linear increase, time $b = 0.0657$, $p = 0.017$, and greater deceleration, time$^2$ $b = -0.0045$, $p = 0.038$). Having no stressful life events in the past 3 months before internship, compared to having at least one life stressor (e.g., getting married, having a child, death of family member, financial loss, etc.), predicted lower pre-internship PHQ-9 levels, intercept, $b = -0.2015$, $p = 0.014$, with no effects on trajectory from pre-internship levels (all $ps > 0.20$). Participants who took more days off in the past month (as indicated in quarterly surveys) had an overall flatter depressive symptom profile during internship, time, $b = -0.0130$, $p = 0.017$, time$^2$ $b = 0.0009$, $p = 0.034$. Cohort, pre-internship stressful life events, and mean days off in the past month were controlled for in the GCM model reported next.

### 3.4. Associations between hair cortisol and depressive symptom responses to internship stress

Though hair cortisol and depressive symptoms both increased during the early months of internship, these changes were not significantly related to each other ($r(65) = -0.08$, $p = 0.51$). The lack of relationship was confirmed using growth curve modeling to test the association between hair cortisol change with depressive symptom trajectory. Specifically, while controlling for identified covariates and

the association of pre-internship hair cortisol with pre-internship depressive symptoms, the change in hair cortisol from pre-internship to 2 months was not associated with depressive symptom trajectory during internship (hair cortisol change-by-time $b = 0.0323$, $p = 0.286$, hair cortisol change-by-time$^2$ $b = -0.0021$, $p = 0.378$). In sum, though cortisol and depressive symptoms both increased with stressor exposure, these appear to have been independent phenomena, not directly associated with each other.

### 3.5. Posthoc analyses

We assessed whether factors that predicted depressive responses also predicted cortisol responses. Hair cortisol response to stressor onset did not correlate with increases in work hours, decreases in sleep, and changes in subjective/emotional stress responses, such as increases in perceived stress or loss of mastery/control (all $ps > 0.20$). However, these variables did correlate with depressive symptom increase (increased work hours: $r(65) = 0.349$, $p = 0.004$; decreased sleep: $r(51) = 0.386$, $p = 0.004$; increased perceived stress: $r(50) = 0.394$, $p = 0.004$; decreased mastery/control: $r(51) = 0.293$, $p = 0.033$).

## 4. Discussion

This study examined the relationships between chronic stress, hair cortisol, and depressive symptoms, longitudinally following interns over the course of the "standard" predictable stress of medical internship year. We confirmed prior work (Mata et al., 2015; Sen et al., 2010) showing increased depression symptoms in response to this challenging year, and prospectively documented a striking increase in hair cortisol levels in its first few months of internship. However, we found no relationship between neuroendocrine and depressive responses to stress exposure, suggesting that changes in these two dimensions reflect different aspects of stress reactivity.

Both neuroendocrine and depressive symptoms increased with the chronic stressor of medical internship. Elevated hair cortisol levels in stressed populations have previously been found (reviewed in Stalder et al., 2017; Staufenbiel et al., 2013). This study expands prior literature by *prospectively* examining *changes* in hair cortisol through the onset and unfolding of exposure to a substantial, prolonged stressor. Results show an initial sharp increase with stressor onset, providing prospective validation of the hair cortisol method as a field-friendly biological marker of stress exposure—a finding consistent with relocation studies in primates (e.g., Davenport et al., 2006) and one prospective human study that examined hair cortisol concentrations pre/post (but not throughout) military deployment (Steudte-Schmiedgen et al., 2015). Our data also show recovery, despite ongoing challenges, and a rise back to pre-internship levels as transition to the second residency year approached. Like neuroendocrine responses, depressive symptoms also increased with stressor onset. However, in contrast to cortisol, they remained elevated throughout internship—consistent with a larger previous study of medical internship (Sen et al., 2010) and meta-analytic evidence (Mata et al., 2015).

Despite an increase in both hair cortisol and depressive symptoms with stressor onset, the two measures clearly followed distinct trajectories and were not directly correlated with each other. Our results are consistent with a recent meta-analysis that found no clear relationships between hair cortisol and self-reported depressiveness (Stalder et al., 2017). Links between HPA axis hyperactivity and depression have been shown in studies using cortisol measures in blood, saliva, and urine (reviewed in Nemeroff and Vale, 2005), but great variability exists, depending on patient characteristics and clinical status or subtype (Lamers et al., 2013; Stetler and Miller, 2011). Recent scientific developments suggest that some previously described HPA axis abnormalities in depression may reflect the impact of shared underlying vulnerability factors (e.g., genetic heritage interacting with developmental experiences) rather than direct HPA-depression links per se (Baumeister et al., 2014). There were no clear and consistent linkages between cortisol levels and depressive symptoms in our study. These longitudinal data, collected in the context of a chronic stressor that triggers new onset depression in a substantial number of those exposed (Mata et al., 2015; Sen et al., 2010), add weight to the idea that hypercortisolemia is neither a cause nor direct correlate of depressive symptom states, at least in the context of a non-clinical sample. Replication is needed in longitudinally followed samples using validated tools for assessing onset of clinical disorders.

The decoupled nature of the hair cortisol and depressive symptom response suggests that these measures reflect different aspects of stress responsivity. Depressive symptom elevations with stress were closely related to external stressor demands (e.g., increased work hours, shorter sleep) and stress perceptions (increased perceived stress, loss of mastery/control), which has been extensively demonstrated in the literature. For example, close relationships have been shown between depressive symptoms and perceived stress (Cohen et al., 1983), long working hours (> 55 h/week; Virtanen et al., 2011), sleep disturbances (Ford and Cooper-Patrick, 2001), and loss of mastery/control (Marshall and Lang, 1990). Hair cortisol also increased with stressor onset, but unlike depressive symptom responses, it did not correspond to reported stressor demands or subjective/emotional stress responses, such as increases in perceived stress. The lack of covariance between subjective stress and HPA axis activity is consistent with prior studies of acute (salivary/plasma) HPA measures (Dickerson and Kemeny, 2004; Mayer et al., 2017), and also in descriptive and meta-analytic reviews of hair cortisol studies (Stalder et al., 2017; Staufenbiel et al., 2013), although exceptions exist (Oldehinkel et al., 2011; Oswald et al., 2004; Schlotz et al., 2008). In contrast to subjective emotional distress, cortisol is known to more clearly track things like stressor anticipation, novelty/familiarity, and social-evaluative threat (Curtis et al., 1976; Davis et al., 1981; Dickerson and Kemeny, 2004; Gaab et al., 2005; Mayer et al., 2017; Peters et al., 2011). Such factors may have been in play here. Our repeated sampling revealed a striking rise during the first few months of stressor exposure, a nadir (below "baseline") in the 10-month samples, and a return to pre-internship levels at the end of the year. This pattern suggests that hair cortisol may respond to specific psychological features of the stress context. The initial hair cortisol increase could reflect the joint impact of novelty and social evaluative threat. Novel medical settings, staff, and procedures posing many types of challenges may have increased hair cortisol during the first few months of internship. Constant scrutiny by peers and senior physicians may be a potent social-evaluative threat that may well have contributed to this initial rise. Repeated exposure to challenge and once novel stimuli (Davis et al., 1981; Peters et al., 2011) and repeated exposure to social-evaluative threat (Pruessner et al., 1997; Schommer et al., 2003), lead to loss of this initial HPA reactivity over time. Our study shows a striking decline in cortisol levels after the initial months of internship, perhaps related to this accommodation process. However, levels rise again as transition to second year approaches. Anticipation of unfamiliar challenge is a well-established activator of the HPA axis (Gaab et al., 2005), which may explain the end rise and the comparable "elevations" that were present prior to internship start and prior to transition to residency. Further work is needed to more directly test these speculative interpretations. We did not use established measures that assess perceptions of stressor context, related to anticipation, novelty/familiarity, and social-evaluative threat, but such measures should be included in future studies.

### 4.1. Strengths and limitations

The study had several strengths. It used a prospective and longitudinal design in the naturalistic, yet standardized, chronic stress setting of medical internship—an established model of stress and depression (Sen et al., 2010). It also assessed cortisol concentrations repeatedly over time using hair samples, allowing us to trace changes

over time more thoroughly than ever before. The study also had limitations. The sample size was small and replication with a larger sample is needed. This will be more feasible if self-collection hair protocols are employed in future studies (e.g., see Gow et al., 2011). A larger sample size would also allow us to examine depressive symptom sub-clusters (e.g., emotional, cognitive, or vegetative symptoms) and their links to hair cortisol. Medical interns with diverse specialties were included to examine common links between stress, hair cortisol, and depressive symptoms across specialties. We did not have enough power to test if specialties differed in psychobiological growth trajectories. However, a recent meta-analysis of studies recruiting residents from both single (n = 26) and multiple (n = 28) specialties found that the prevalence of depressive symptoms was similar across specialties, suggesting common underlying factors (Mata et al., 2015). Nevertheless, variability in stress likely exists between specialties and future studies may only include a few specialties so that subgroup analyses are feasible. Another limitation is that our hair cortisol analyses assumed a hair growth rate of 1 cm/month, but individual variability in hair growth rates can be quite large (Schütz et al., 1993), which could have impacted our results.

Furthermore, it is possible that seasonal changes affected hair cortisol levels. Hair cortisol concentrations were highest during the first 4 months of internship, which also coincided with summer-fall months (July through October). In the absence of a control group, this leaves the internship effect potentially confounded by a season effect. However, existing literature does not clearly demonstrate season effects that could explain the early rise in hair cortisol seen here. Some studies do report higher hair cortisol concentrations in summer months (Braig et al., 2015; Fischer et al., 2017; Staufenbiel et al., 2015), but a large-scale cohort sample (Whitehall II) found the opposite effect, showing higher HCC in samples collected in the winter (Abell et al., 2016). Studies examining seasonal patterns in salivary and urinary cortisol secretion also showed lower cortisol during summer months and higher levels in the winter (Hansen et al., 2001; Persson et al., 2008). Seasonal variation in hair cortisol might be explained, at least in part, by light/sunshine exposure or transpiration (proposed by Braig et al., 2015; Gao et al., 2014). Hair samples exposed to artificial ultraviolet (UV) radiation or natural sunlight show reduced cortisol concentrations (Li et al., 2012; Wester et al., 2016), consistent with the studies showing reduced levels in the summer. Hair cortisol levels are not altered following sweat-inducing interventions (Grass et al., 2015). In sum, large-scale observational evidence and experimental work do not support the idea that higher hair cortisol levels from July to October reflected the impact of summer season or sunlight exposure. However, a seasonal impact on hair cortisol results cannot be fully ruled out in this study and requires further research. Also, though our prospective and longitudinal design improved on previous cross-sectional studies in allowing us to examine changes across the internship year in psycho-biological measures, the linkages reported are correlational and causal insights require controlled laboratory work.

Cohort effects in hair cortisol and depressive symptoms present another limitation. Internship and assay procedures did not change over time, but it is possible that assay technology became more sensitive. It is also possible that cohorts differed in resilience, perhaps reflecting random variations given the small cohort sizes. Follow-up work with an enlarged sample is needed. Another limitation is that depressive symptoms were only assessed using symptom self-reports. Diagnostic validity of the PHQ-9 is good and comparable to clinician-administered assessments (Kroenke and Spitzer, 2002; Kroenke et al., 2001; Spitzer et al., 1999), but we were not evaluating depression using clinical interviews and DSM criteria to make diagnoses. Also, we do not know if depression onset examined here is comparable to the development of depression types examined in earlier studies (e.g., melancholic depression).

### 4.2. Summary and conclusions

Studies over the past 50 years have sometimes documented HPA axis hyperactivity in depression, but the precise nature of the relationship between this system and major depressive disorder remains uncertain. Prospective, longitudinal tracking of HPA activity over time in an established stress context allowed us to explore its potential links to depressive symptom development in a new way. Our results showed a sharp increase in hair cortisol with internship onset, reflecting stress exposure, but this rise did not predict depressive symptom development during internship stress, despite clear rise in depressive symptoms as well. Instead, hair cortisol and depressive responses were decoupled in the context of chronic stress exposure, following different trajectories through the year − depressive symptoms were elevated throughout internship and correlated with stressor demands and subjective stress reactions; in contrast, hair cortisol fluctuated through the year, perhaps tracking specific contextual aspects related to anticipation, novelty/familiarity, and social evaluative threat. These findings suggest that the HPA axis may not be mechanistically linked with the development of depressive symptoms in the context of stress, at least within this naturalistic stress paradigm.

Our results suggest two main future directions. First, the field has spent decades looking for direct HPA-depression links, but such direct associations have been inconsistent in both clinical and non-clinical populations. Our data support the idea that the link between HPA activity and depressive states may not be direct and causal, at least in non-clinical samples, and this needs further exploration in similar, longitudinal studies that can track new onsets of DSM-defined disorder in the context of stress with careful measurement of HPA axis variables. Our data are congruent with newer research developments that point towards shared underlying vulnerability factors, rather than direct HPA-depression links (Baumeister et al., 2014). Genetic factors, in interaction with early developmental experiences, likely shape both vulnerability to depression in the context of stress as well as alterations in HPA regulatory “set points.” Understanding person-environment interactions that mold vulnerable phenotypes will be the next frontier in depression research, and the HPA axis system—as a critical, adaptational system that is sensitive to the stress environment and shaped both genetically and epigenetically—may offer unique insights.

Second, links between “stress” and HPA axis functioning need to be clarified. Stress itself is a complex construct that includes stress exposure, perceptions of stress, and bio-psycho-social responses to stress. In our study, the HPA axis did not respond to increased stressor demands or stress perceptions, which were present throughout internship, but it instead appeared to reflect reactivity to specific contextual features, perhaps related to anticipation, novelty/familiarity, and social evaluative threat. Understanding what aspects of “stress” activate the HPA axis, both in the context of acute and chronic stress, will be important in clarifying its role in stress-related diseases (Mayer et al., 2017).

Our innovative design can be a model for future work. Using new technology that captures longer term HPA axis functioning within a longitudinal and prospective model that takes advantage of a naturalistic “experiment” will provide a useful context for examining links between stress, HPA axis functioning, and depression in future, larger studies.

## Conflict of interest

None of the authors have any actual or potential conflicts of interest related to the findings of this study.

## Role of the funding source

This work was supported by the National Institute of Mental Health (R01-MH-101459) and the Inaugural Oscar Stern Award granted to

Srijan Sen, as well as the Blue Cross Blue Shield of Michigan Foundation (1989.SAP) and the University of Michigan Rackham Graduate School awards (Rackham Pre-candidate Student Research Grant; Rackham International Student Fellowship) granted to Stefanie Mayer.

## Contributors

Stefanie Mayer conceived the study, and led design development, data collection, data analysis, and interpretation. She wrote the first draft of the manuscript and was instrumental in editing the manuscript. Drs. Nestor L. Lopez-Duran, Srijan Sen, and James L. Abelson provided critical guidance and input for the conception and design of the study, data analysis and interpretation, as well as manuscript editing. All authors contributed to and have approved the final manuscript.

## Acknowledgements

The authors thank Elena Frank, Ph.D. for her skilled assistance in data collection, and Joan Zhao, M.A., M.S. for data management.

## References

Abell, J.G., Stalder, T., Ferrie, J.E., Shipley, M.J., Kirschbaum, C., Kivimaki, M., Kumari, M., 2016. Assessing cortisol from hair samples in a large observational cohort: the Whitehall II study. Psychoneuroendocrinology 73, 148–156.

Adam, E.K., Kumari, M., 2009. Assessing salivary cortisol in large-scale, epidemiological research. Psychoneuroendocrinology 34, 1423–1436.

Adam, E.K., Doane, L.D., Zinbarg, R.E., Mineka, S., Craske, M.G., Griffith, J.W., 2010. Prospective prediction of major depressive disorder from cortisol awakening responses in adolescence. Psychoneuroendocrinology 35, 921–931.

Baumeister, D., Lightman, S.L., Pariante, C.M., 2014. The interface of stress and the HPA axis in behavioural phenotypes of mental illness. Curr. Top. Behav. Neurosci. 18, 13–24.

Braig, S., Grabher, F., Ntomchukwu, C., Reister, F., Stalder, T., Kirschbaum, C., Genuneit, J., Rothenbacher, D., 2015. Determinants of maternal hair cortisol concentrations at delivery reflecting the last trimester of pregnancy. Psychoneuroendocrinology 52, 289–296.

Butterfield, P.S., 1988. The stress of residency: a review of the literature. Arch. Intern. Med. 148, 1428–1435.

Cohen, S., Kamarck, T., Mermelstein, R., 1983. A global measure of perceived stress. J. Health Soc. Behav. 24, 385–396.

Cooper, G.A.A., Kronstrand, R., Kintz, P., 2012. Society of hair testing guidelines for drug testing in hair. Forensic Sci. Int. 218, 20–24.

Curtis, G.C., Buxton, M., Lippman, D., Nesse, R., Wright, J., 1976. Flooding in vivo during the circadian phase of minimal cortisol secretion: anxiety and therapeutic success without adrenal cortical activation. Biol. Psychiatry 11, 101–107.

Davenport, M.D., Tiefenbacher, S., Lutz, C.K., Novak, M.A., Meyer, J.S., 2006. Analysis of endogenous cortisol concentrations in the hair of rhesus macaques. Gen. Comp. Endocrinol. 147, 255–261.

Davis, H.A., Gass, G.C., Bassett, J.R., 1981. Serum cortisol response to incremental work in experienced and naive subjects. Psychosom. Med. 43, 127–132.

Dettenborn, L., Muhtz, C., Skoluda, N., Stalder, T., Steudte, S., Hinkelmann, K., Kirschbaum, C., Otte, C., 2012. Introducing a novel method to assess cumulative steroid concentrations: increased hair cortisol concentrations over 6 months in medicated patients with depression. Stress 15, 348–353.

Dickerson, S.S., Kemeny, M.E., 2004. Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. Psychol. Bull. 130, 355–391.

Ehlert, U., Gaab, J., Heinrichs, M., 2001. Psychoneuroendocrinological contributions to the etiology of depression, posttraumatic stress disorder, and stress-related bodily disorders: the role of the hypothalamus–pituitary–adrenal axis. Biol. Psychol. 57, 141–152.

Faresjo, A., Theodorsson, E., Chatziarzenis, M., Sapouna, V., Claesson, H.P., Koppner, J., Faresjo, T., 2013. Higher perceived stress but lower cortisol levels found among young Greek adults living in a stressful social environment in comparison with Swedish young adults. PLoS One 8, e73828.

Fischer, S., Duncko, R., Hatch, S.L., Papadopoulos, A., Goodwin, L., Frissa, S., Hotopf, M., Cleare, A.J., 2017. Sociodemographic, lifestyle, and psychosocial determinants of hair cortisol in a South London community sample. Psychoneuroendocrinology 76, 144–153.

Ford, D.E., Cooper-Patrick, L., 2001. Sleep disturbances and mood disorders: an epidemiologic perspective. Depress. Anxiety 14, 3–6.

Gaab, J., Rohleder, N., Nater, U.M., Ehlert, U., 2005. Psychological determinants of the cortisol stress response: the role of anticipatory cognitive appraisal. Psychoneuroendocrinology 30, 599–610.

Gao, W., Xie, Q., Jin, J., Qiao, T., Wang, H., Chen, L., Deng, H., Lu, Z., 2010. HPLC-FLU detection of cortisol distribution in human hair. Clin. Biochem. 43, 677–682.

Gao, W., Zhong, P., Xie, Q.Z., Wang, H.Y., Jin, J., Deng, H.H., Lu, Z.H., 2014. Temporal features of elevated hair cortisol among earthquake survivors. Psychophysiology 51, 319–326.

Gotlib, I.H., Joormann, J., Minor, K.L., Hallmayer, J., 2008. HPA axis reactivity: a mechanism underlying the associations among 5-HTTLPR, stress, and depression. Biol. Psychiatry 63, 847–851.

Gow, R., Thomson, S., Rieder, M., Van Uum, S., Koren, G., 2010. An assessment of cortisol analysis in hair and its clinical applications. Forensic Sci. Int. 196, 32–37.

Gow, R., Koren, G., Rieder, M., Van Uum, S., 2011. Hair cortisol content in patients with adrenal insufficiency on hydrocortisone replacement therapy. Clin. Endocrinol. 74, 687–693.

Grass, J., Kirschbaum, C., Miller, R., Gao, W., Steudte-Schmiedgen, S., Stalder, T., 2015. Sweat-inducing physiological challenges do not result in acute changes in hair cortisol concentrations. Psychoneuroendocrinology 53, 108–116.

Gunnar, M.R., Talge, N.M., Herrera, A., 2009. Stressor paradigms in developmental studies: what does and does not work to produce mean increases in salivary cortisol. Psychoneuroendocrinology 34, 953–967.

Hansen, A.M., Garde, A.H., Skovgaard, L.T., Christensen, J.M., 2001. Seasonal and biological variation of urinary epinephrine norepinephrine, and cortisol in healthy women. Clin. Chim. Acta 309, 25–35.

Harris, T.O., Borsanyi, S., Messari, S., Stanford, K., Brown, G.W., Cleary, S.E., Shiers, H.M., Herbert, J., 2000. Morning cortisol as a risk factor for subsequent major depressive disorder in adult women. Br. J. Psychiatry 177, 505–510.

Kendler, K.S., Karkowski, L.M., Prescott, C.A., 1999. Causal relationship between stressful life events and the onset of major depression. Am. J. Psychiatry 156, 837–841.

Kessler, R.C., Berglund, P., Demler, O., Jin, R., Merikangas, K.R., Walters, E.E., 2005. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. Arch. Gen. Psychiatry 62, 593–603.

Kirschbaum, C., Tietze, A., Skoluda, N., Dettenborn, L., 2009. Hair as a retrospective calendar of cortisol production—increased cortisol incorporation into hair in the third trimester of pregnancy. Psychoneuroendocrinology 34, 32–37.

Kroenke, K., Spitzer, R.L., 2002. The PHQ-9: a new depression diagnostic and severity measure. Psychiatr. Ann. 32, 509–515.

Kroenke, K., Spitzer, R.L., Williams, J.B., 2001. The PHQ-9: validity of a brief depression severity measure. J. Gen. Intern. Med. 16, 606–613.

Lamers, F., Vogelzangs, N., Merikangas, K.R., de Jonge, P., Beekman, A.T., Penninx, B.W., 2013. Evidence for a differential role of HPA-axis function, inflammation and metabolic syndrome in melancholic versus atypical depression. Mol. Psychiatry 18, 692–699.

Lee, K.J., Carlin, J.B., 2010. Multiple imputation for missing data: fully conditional specification versus multivariate normal imputation. Am. J. Epidemiol. 171, 624–632.

Levine, S., 2000. Influence of psychological variables on the activity of the hypothalamic–pituitary–adrenal axis. Eur. J. Pharmacol. 405, 149–160.

Li, J.F., Xie, Q.Z., Gao, W., Xu, Y.Y., Wang, S., Deng, H.H., Lu, Z.H., 2012. Time course of cortisol loss in hair segments under immersion in hot water. Clin. Chim. Acta 413, 434–440.

Marshall, G.N., Lang, E.L., 1990. Optimism, self-mastery, and symptoms of depression in women professionals. J. Pers. Soc. Psychol. 59, 132.

Mata, D.A., Ramos, M.A., Bansal, N., Khan, R., Guille, C., Di Angelantonio, E., Sen, S., 2015. Prevalence of depression and depressive symptoms among resident physicians: a systematic review and meta-analysis. JAMA 314, 2373–2383.

Mayer, S.E., Snodgrass, M., Liberzon, I., Briggs, H., Curtis, G.C., Abelson, J.L., 2017. The psychology of HPA axis activation: examining subjective emotional distress and control in a phobic fear exposure model. Psychoneuroendocrinology 82, 189–198.

Nemeroff, C.B., Vale, W.W., 2005. The neurobiology of depression: inroads to treatment and new drug discovery. J. Clin. Psychiatry 66, 5–13.

Oldehinkel, A.J., Ormel, J., Bosch, N.M., Bouma, E., Van Roon, A.M., Rosmalen, J.G.M., Riese, H., 2011. Stressed out? Associations between perceived and physiological stress responses in adolescents: the TRAILS study. Psychophysiology 48, 441–452.

Oswald, L.M., Mathena, J.R., Wand, G.S., 2004. Comparison of HPA axis hormonal responses to naloxone vs psychologically-induced stress. Psychoneuroendocrinology 29, 371–388.

Pearlin, L.I., Schooler, C., 1978. The structure of coping. J. Health Soc. Behav. 19, 2–21.

Persson, R., Garde, A.H., Hansen, A.M., Osterberg, K., Larsson, B., Orbaek, P., Karlson, B., 2008. Seasonal variation in human salivary cortisol concentration. Chronobiol. Int. 25, 923–937.

Peters, S., Cleare, A.J., Papadopoulos, A., Fu, C.H., 2011. Cortisol responses to serial MRI scans in healthy adults and in depression. Psychoneuroendocrinology 36, 737–741.

Pruessner, J.C., Gaab, J., Hellhammer, D.H., Lintz, D., Schommer, N., Kirschbaum, C., 1997. Increasing correlations between personality traits and cortisol stress responses obtained by data aggregation. Psychoneuroendocrinology 22, 615–625.

Roberti, J.W., Harrington, L.N., Storch, E.A., 2006. Further psychometric support for the 10- item version of the perceived stress scale. J. Coll. Couns. 9, 135–147.

Russell, E., Koren, G., Rieder, M., Van Uum, S., 2012. Hair cortisol as a biological marker of chronic stress: current status, future directions and unanswered questions. Psychoneuroendocrinology 37, 589–601.

Schütz, H., Ahrens, B., Erdmann, F., Rochholz, G., 1993. Nachweis von Arznei-und anderen Fremdstoffen in Haaren. Pharm Unserer Zeit 22, 65–78.

Schlotz, W., Kumsta, R., Layes, I., Entringer, S., Jones, A., Wüst, S., 2008. Covariance between psychological and endocrine responses to pharmacological challenge and psychosocial stress: a question of timing. Psychosom. Med. 70, 787–796.

Schommer, N.C., Hellhammer, D.H., Kirschbaum, C., 2003. Dissociation between reactivity of the hypothalamus-pituitary-adrenal axis and the sympathetic-adrenal-medullary system to repeated psychosocial stress. Psychosom. Med. 65, 450–460.

Sen, S., Kranzler, H.R., Krystal, J.H., Speller, H., Chan, G., Gelernter, J., Guille, C., 2010. A prospective cohort study investigating factors associated with depression during medical internship. Arch. Gen. Psychiatry 67, 557–565.

Spitzer, R.L., Kroenke, K., Williams, J.B., 1999. Validation and utility of a self-report

version of PRIME-MD: the PHQ primary care study. JAMA 282, 1737–1744.
Stalder, T., Steudte, S., Miller, R., Skoluda, N., Dettenborn, L., Kirschbaum, C., 2012. Intraindividual stability of hair cortisol concentrations. Psychoneuroendocrinology 37, 602–610.
Stalder, T., Tietze, A., Steudte, S., Alexander, N., Dettenborn, L., Kirschbaum, C., 2014. Elevated hair cortisol levels in chronically stressed dementia caregivers. Psychoneuroendocrinology 47, 26–30.
Stalder, T., Steudte-Schmiedgen, S., Alexander, N., Klucken, T., Vater, A., Wichmann, S., Kirschbaum, C., Miller, R., 2017. Stress-related and basic determinants of hair cortisol in humans: a meta-analysis. Psychoneuroendocrinology 77, 261–274.
Staufenbiel, S.M., Penninx, B.W., Spijker, A.T., Elzinga, B.M., van Rossum, E.F., 2013. Hair cortisol, stress exposure, and mental health in humans: a systematic review. Psychoneuroendocrinology 38, 1220–1235.
Staufenbiel, S.M., Penninx, B.W., de Rijke, Y.B., van den Akker, E.L., van Rossum, E.F., 2015. Determinants of hair cortisol and hair cortisone concentrations in adults. Psychoneuroendocrinology 60, 182–194.
Stetler, C., Miller, G.E., 2011. Depression and hypothalamic-pituitary-adrenal activation: a quantitative summary of four decades of research. Psychosom. Med. 73, 114–126.
Steudte-Schmiedgen, S., Stalder, T., Schönfeld, S., Wittchen, H.-U., Trautmann, S., Alexander, N., Miller, R., Kirschbaum, C., 2015. Hair cortisol concentrations and cortisol stress reactivity predict PTSD symptom increase after trauma exposure during military deployment. Psychoneuroendocrinology 59, 123–133.
Taylor, S.E., Repetti, R.L., Seeman, T., 1997. Health psychology: what is an unhealthy environment and how does it get under the skin? Annu. Rev. Psychol. 48, 411–447.
Tyrka, A.R., Wier, L., Price, L.H., Ross, N., Anderson, G.M., Wilkinson, C.W., Carpenter, L.L., 2008. Childhood parental loss and adult hypothalamic-pituitary-adrenal function. Biol. Psychiatry 63, 1147–1154.
Virtanen, M., Ferrie, J.E., Singh-Manoux, A., Shipley, M.J., Stansfeld, S.A., Marmot, M.G., Ahola, K., Vahtera, J., Kivimäki, M., 2011. Long working hours and symptoms of anxiety and depression: a 5-year follow-up of the Whitehall II study. Psychol. Med. 41, 2485–2494.
WHO, 2017. Depression and Other Common Mental Disorders: Global Health Estimates. World Health Organization, Geneva, Switzerland.
Wennig, R., 2000. Potential problems with the interpretation of hair analysis results. Forensic Sci. Int. 107, 5–12.
Wester, V.L., van der Wulp, N.R., Koper, J.W., de Rijke, Y.B., van Rossum, E.F., 2016. Hair cortisol and cortisone are decreased by natural sunlight. Psychoneuroendocrinology 72, 94–96.
Wikenius, E., Moe, V., Kjellevold, M., Smith, L., Lyle, R., Waagbo, R., Page, C.M., Myhre, A.M., 2016. The association between hair cortisol and self-reported symptoms of depression in pregnant women. PLoS One 11, e0161804.
Wilcox, R., 1998. Trimming and winsorization. In: Armitage, P., Colton, T. (Eds.), Encyclopedia of Biostatistics. Wiley, Chichester, England, pp. 4588–4590.
Xie, Q., Gao, W., Li, J., Qiao, T., Jin, J., Deng, H., Lu, Z., 2011. Correlation of cortisol in 1-cm hair segment with salivary cortisol in human: hair cortisol as an endogenous biomarker. Clin. Chem. Lab. Med. 49, 1–7.